

Application No. 10/590,118
Paper Dated: February 6, 2009
In Reply to USPTO Correspondence of January 6, 2009
Attorney Docket No. 4544-062454

AMENDMENTS TO THE CLAIMS

Claims 1-10 (cancelled).

Claim 11 (currently amended): A diagnostic kit for detecting pulmonary and extra pulmonary tuberculosis, comprising a test card "TB Screen" coated with a hydrophobic material, mixing sticks comprising a glycolipid from a *Mycobacterium tuberculosis H₃₇RV* an antigen suspension intercalated or coupled with a liposome surface, a positive control comprising an anti-mycobacterial glycolipid antibody from *Mycobacterial tuberculosis*, and a negative control comprising serum antibodies from a subject not previously exposed to *Mycobacterial tuberculosis*.

Claim 12 (previously presented): The kit as claimed in claim 11, wherein said antigen suspension is a liposome antigen and said test card is a plastic slide.

Claim 13 (previously presented): The kit as claimed in claim 11, wherein said negative control is prepared from the blood of a normal young rabbit.

Claim 14 (previously presented): The kit as claimed in claim 11, wherein said positive control is prepared from a 4 to 6 month old rabbit which is immunized with mycobacterium antigens and bled periodically.

Claim 15 (currently amended): A method for testing individuals for of detecting tuberculosis using a kit comprising the steps of applying a positive control, a negative control and a test sample to, each in circular motion on a test card coated with a hydrophobic material, wherein said positive control is an anti-mycobacterial glycolipid antibody from *Mycobacterial tuberculosis*, and wherein said negative control are serum antibodies from a subject not previously exposed to *Mycobacterial tuberculosis*; adding an antigen suspension to said each of the positive, said negative and test said sample; and interpreting a result to interpret the results, wherein clumping of a specific antigen in the suspension and an antibody is observed

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as dark blue agglutination in the positive control and the test sample is prognostic for which contains the an active tuberculosis infection.

Claim 16 (previously presented): The method as claimed in claim 15, wherein said antigen suspension is a liposome antigen.

Claim 17 (currently amended): The method as claimed in claim 16, wherein said the lipid antigen for positive control is prepared comprising the steps of:

growing Mycobacterium tuberculosis Mycobacterium tuberculosis H₃₇Rv (ATCC-27294) strain on Sautons media;

harvesting cells in the media by centrifugation at 4° to 10°C;
subjecting said cells to the step of sonication;
extracting the antigens from said cells;
adding chloroform and methanol mixture (2:1) to said antigens with stirring at room temperature; and

subjecting the mixture to the step of filtration, thereby forming a suspension;
separating wherein the said suspension thus obtained is transferred into a
separating funnel and kept overnight until two distinct layers are separated, an upper aqueous
phase and is removed and the a lower organic phase;

removing said upper aqueous phase; retained after filtration,
drying said organic phase, being dried by evaporating the thereby forming a
solvent containing to obtain the a lipid; and
purifying subjecting said lipid to the further step of purification.

Claim 18 (currently amended): The method as claimed in claim 15, wherein said antigen suspension is prepared comprising the steps of:

adding a phosphatidylcholine, a cholesterol, a lipid antigen and a dye in a
flask, thereby forming a solvent layer; and

evaporating the said solvent layer, thereby forming dried contents in a vacuum
evaporator;

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dissolving the said dried contents thus obtained in absolute alcohol at 4° to 10°C for 1 to 2 hours to produce the said antigen suspension;

adding said antigen suspension to a sucrose solution; with continuous stirring and keeping said suspension

maintaining a temperature of at 2° to 8°C overnight;

subjecting said suspension to centrifugation, thereby forming a supernatant and a pellet; and

discarding the said supernatant; and

suspending the said pellet obtained into in a buffer and stirring the same at 4° to 10°C.

Claim 19 (previously presented): The method as claimed in claim 16, wherein said lipid antigen is further purified using column chromatography.

Claim 20 (previously presented): The method as claimed in claim 18, wherein said buffer comprises NaH₂PO₄·2H₂O, KH₂PO₄, EDTA, Choline Chloride and Thiomersol.

Claim 21 (currently amended): The method as claimed in claim 18, wherein said dye is Sudan Black black B or Sudan red in chloroform.

Claim 22 (new): The method as claimed in claim 15, wherein said anti-mycobacterial glycolipid antibody is isolated from a rabbit immunized against a purified glycolipid antigen from *Mycobacterium tuberculosis* H₃₇Rv.

Claim 23 (new): The method as claimed in claim 15, wherein said antibodies from a subject not previously exposed to *Mycobacterial tuberculosis* are isolated from a rabbit that has not been exposed to *Mycobacterial tuberculosis*.

Claim 24 (new): The method as claimed in claim 15, wherein said anti-mycobacterial glycolipid antibody is coupled onto a surface of a liposome.

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Claim 25 (new): The method as claimed in claim 23, wherein said rabbit was immunized against a heat inactivated sonicated *Mycobacterium tuberculosis* H37Rv strain.